IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

App. No.	:	10/699,512	Confirmation No. 3570
Applicant	:	Bennett, G.N.	
Filed	:	October 31, 2003	
TC/A.U.	:	1637	
Examiner	:	Fredman, J.N.	
Docket No.	:	31175413-003002	
Customer No.	:	51738	
Entitled	:	RECOMBINATION ASSEMBLY OF LARGE DNA FRAGMENTS	

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF GEORGE N. BENNETT UNDER 37 CFR § 1.131

I, George N. Bennett, Declare as follows:

I am at least 18 years of age and am competent in all respects to make the following statements.

1. I am the sole inventor for claims 1-8 currently pending in US Patent Application No. 10/699,512.

2. The work presented in US Patent Application No. 10/699,512 was conceived prior to October 31, 2001.

3. Although the dates have been redacted, the attached laboratory PowerPoint presentation (Exhibit A) demonstrates the conception or practice of the invention prior to October 31, 2001.

4. Although the dates have been redacted, the attached laboratory notebook (Exhibit B) demonstrates the conception or practice of the invention prior to October 31, 2001.

I further declare that all statements made herein of my own knowledge are true and made on information believed to be true; further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of any application for which it is used.

Dated: Aug 18, 2006

Respectfully submitted,

By George Bennett

Dr. George N. Bennett, Ph.D.
Department Chair
Dept. of Biochemistry and Cell Biology
Rice University
Houston, TX

EXHIBIT A

Chromosomal integration of large designer DNA into *E. Coli*

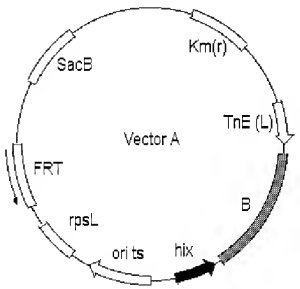
[redacted]

[redacted]

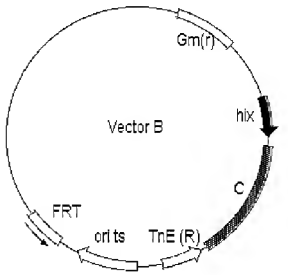
Figure 1. Components for DNA Integration

Receptor Strain

Vector A



Vector B



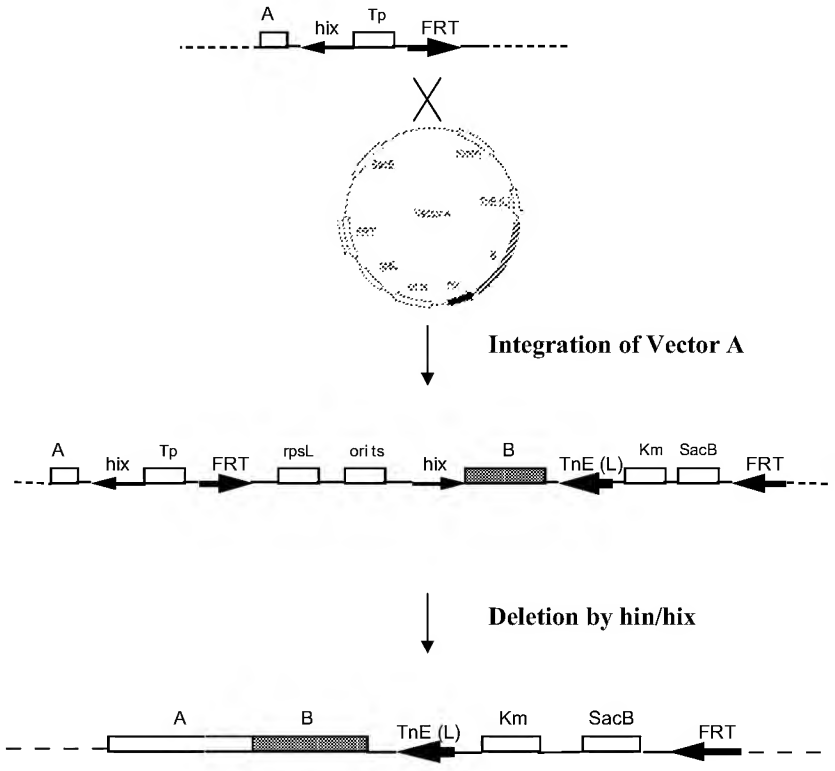
Integration/Excision:
Hix/hin
FRT/Flp
Tn1514- TnE(R/L)

Counter-selection:
rpsL streptomycin sensitivity
SacB sucrose sensitivity

Antibiotic Selection:
Km kanamycin resistance
Tp trimethopin resistance
Gm gentamycin resistance

Plasmid loss selection:
ori ts temperature senstive replicon

Figure 2. Integration and Excision Scheme



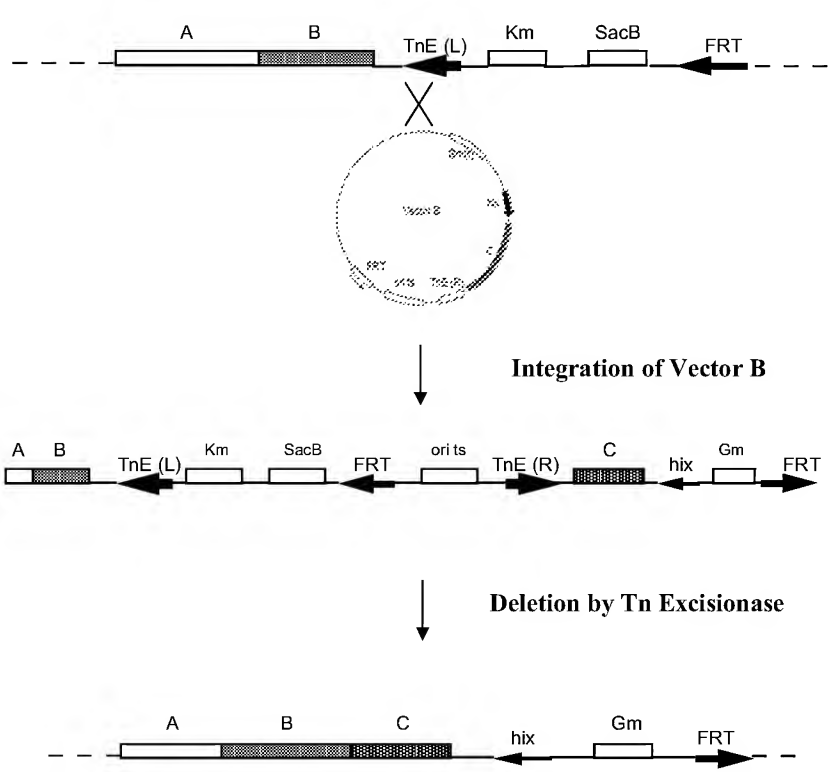
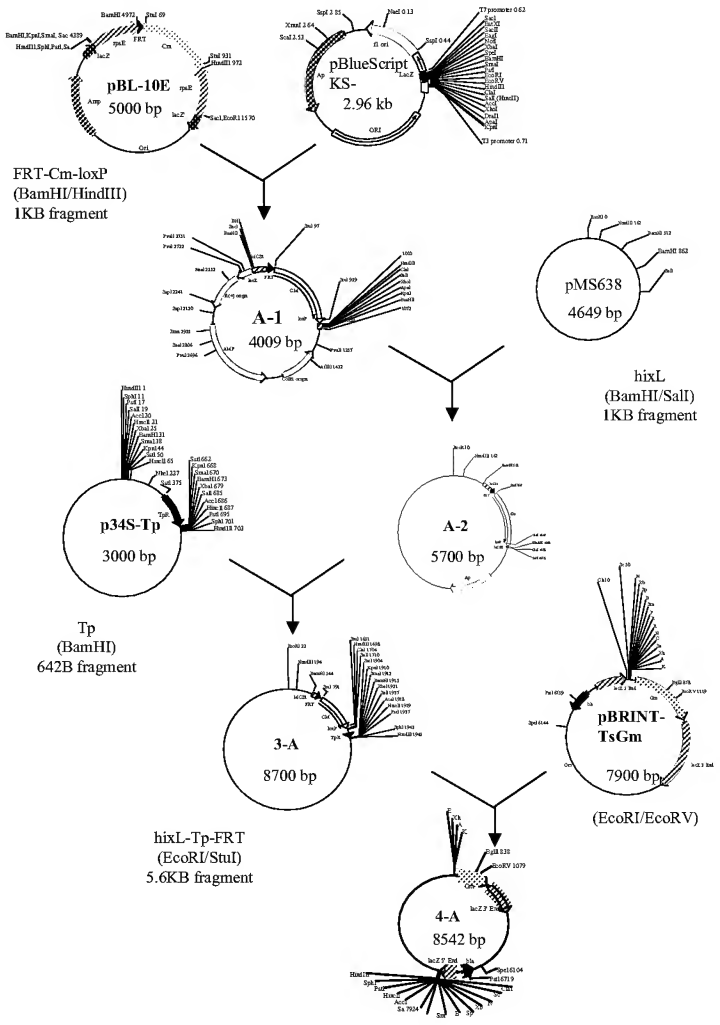


Figure 3. Construction of Receptor Strain

Susy McKay



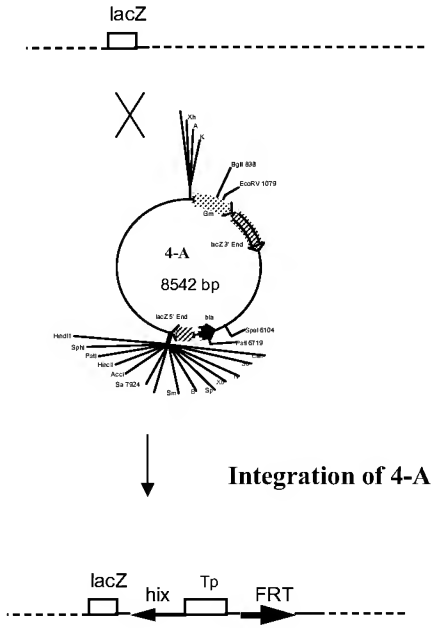
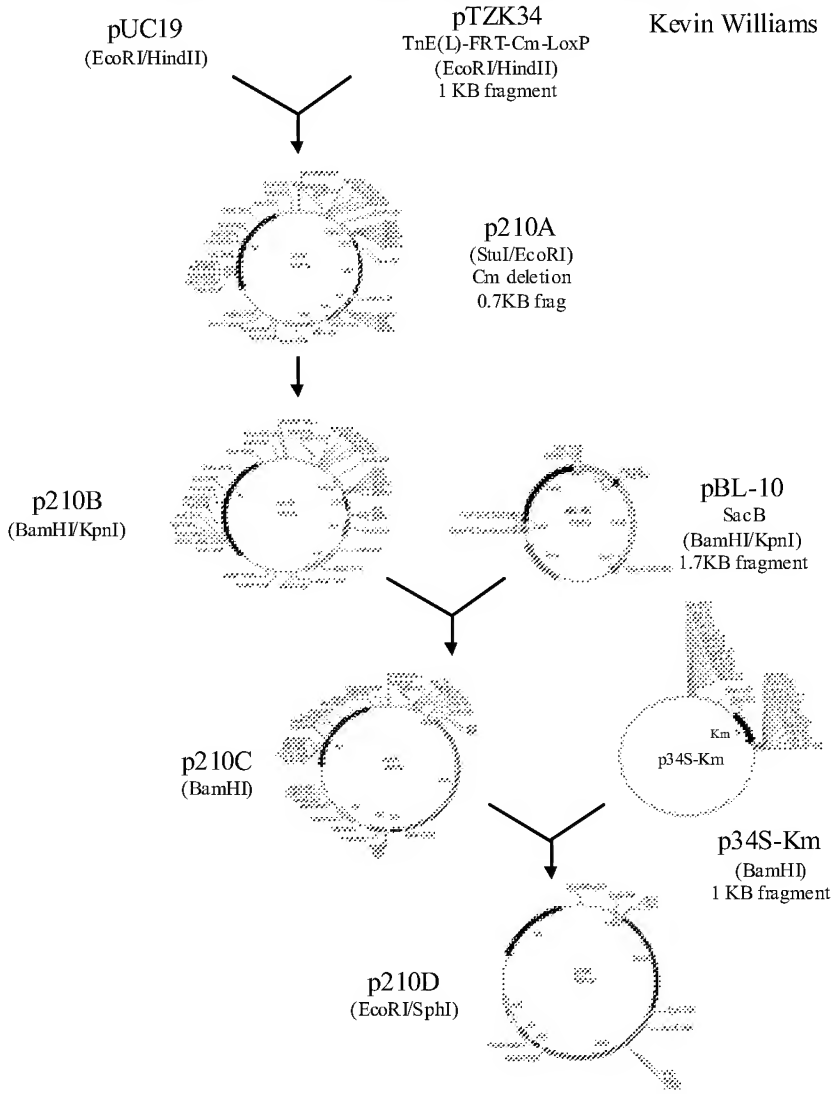


Figure 4. Construction of Vector A



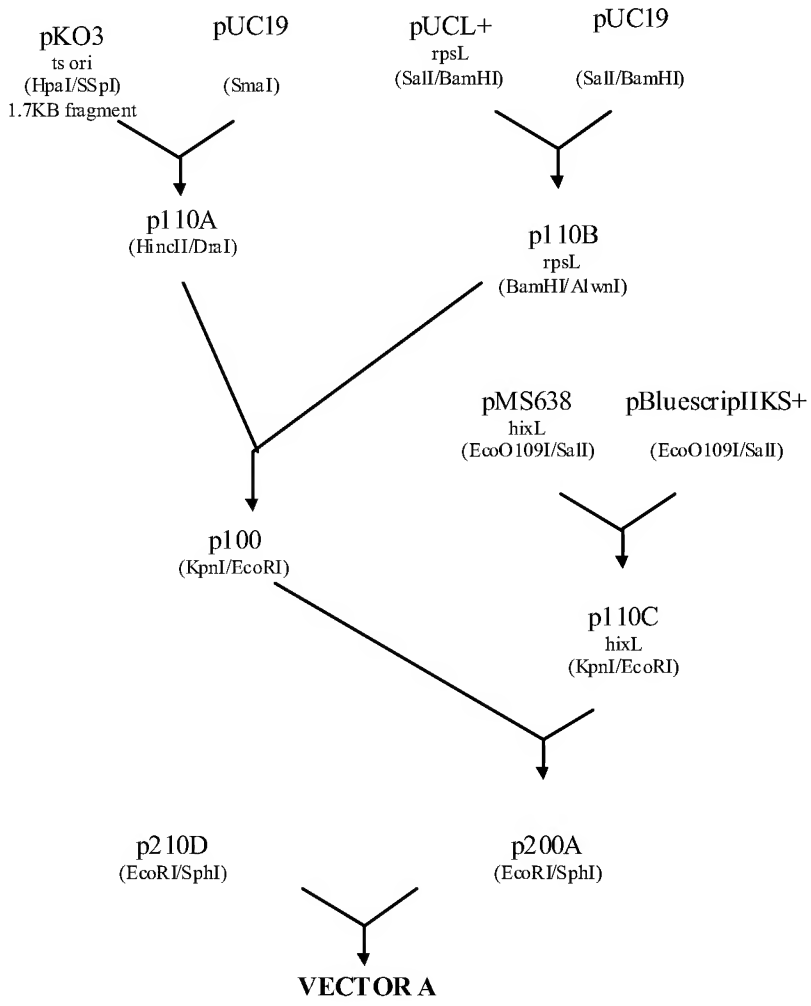


Figure 5. Construction of Vector B

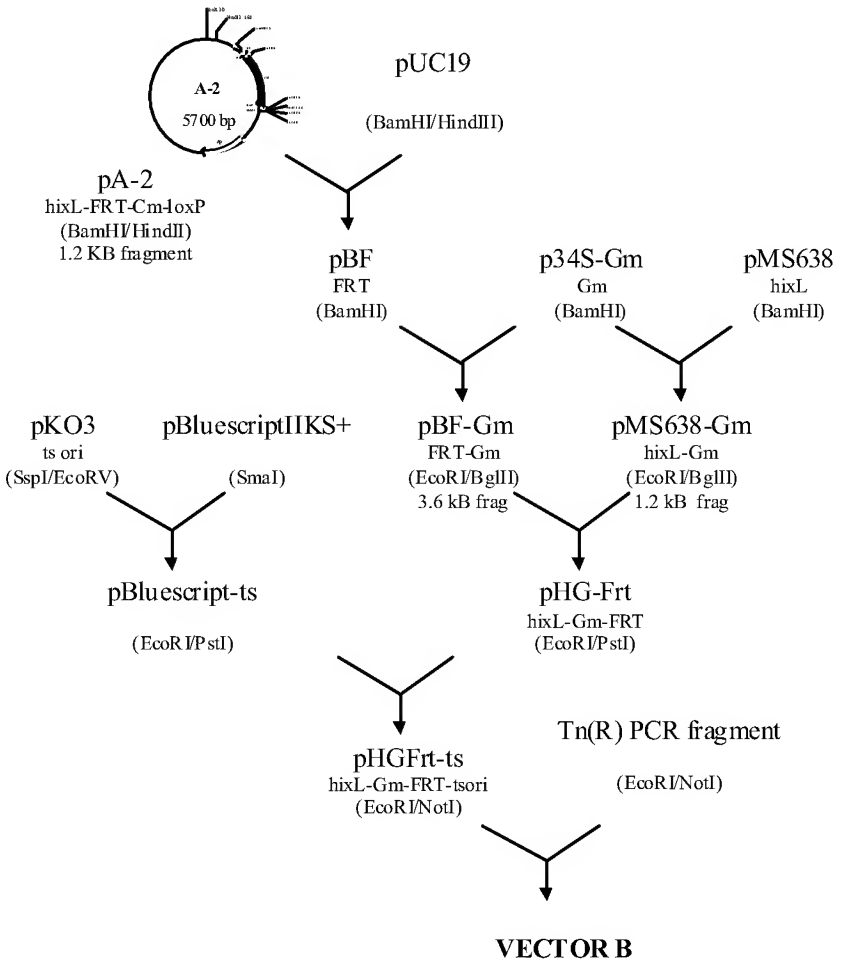
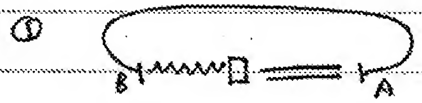
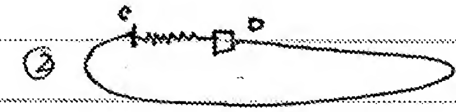


EXHIBIT B

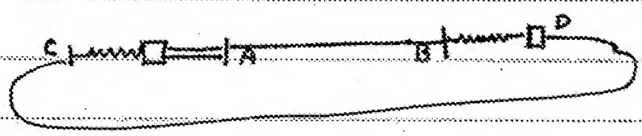


region AB cloned into plasmid ①
Specific recomb site ▯ (eg FLP yeast)

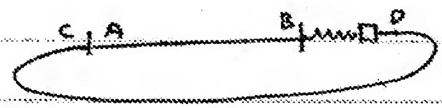
~~~ = conjugative transposon seq, (eg Tn916) :
selection markers,
replication functions as desired



② recipient plasmid or chromosome DNA
bearing 1/2 transposon seq and ▯



double X insert at ▯
made by selection for marker =



removal of conj transposon
precise excision (eg Tn916)
+ selection by loss of
gene at =

reiterate with successive
versions of ①

joining of fragments AB, CD
at specific junction without depending
on sequence at end or within segments

control of FLP or transposon excision
could be by regulation of amt of amount of protein
present in host (eg by regulated expression)

if use two different conj transposons
can go with addition to
either end & switch
back & forth

SIGNATURE
George Bennett

DATE
REDACTED

DISCLOSED TO AND UNDERSTOOD BY
Kaolee Sh

DATE

WITNESS

DATE
~~~